March 11, 1983

see postcard 830309

It has been forty years since I began as a young student in my own research in what we now call molecular biology, with the late Francis Ryan. It has been twenty-five years since I first began to write extensively about the human importance of molecular genetic research, the idea that there could be what we would today call a genetic engineering technology. Some time later I felt that perhaps my correct role and the greatest service as part of this program was to share with the colleagues who are working today in the laboratory and with a wider audience some of my own reflections over this period of time.

Any symposium or lecture on the future has implications of prophecy. We know that to be a prophet often is a somewhat dangerous profession, because the prophet pretends to foretell the future and the prophet also tries to help make the future, and in the very discussion as to which are the possibilities that lie before us, there must be some effort to guide those choices. We make the future and we cannot so much foretell it as to try to guide it and direct it. I think it is of the utmost importance that there be a wide understanding of the technological possibilities that are in front of us today to try to afford the most useful direction to these very powerful and very exciting technologies so that they can be of the most human advantage. But let me first remind you that this subject has a history; it does not emerge fully grown like Venus out of the clam shell. It has an origin; it has a history; there are methods that have been used in genetic engineering over some period of time. Different methods are used for different purposes.

Let's first have a look at the history of genetics. The subject can be well said to have begun in 1865 when the monk Gregor Mendel in Brno did his famous experiments on the crossbreeding of the garden pea, and for the first time constructed the conception of independent factors in heredity - what we now call the different genes. His approach was an abstract one, based entirely on a mathematical analysis of the different kinds of progeny that were produced when plants of different characteristics were cross bred to one another. He could have had no concept as to the material basis of these anlage, of what we now call the genes, and for many years genetics had many productive consequences without an understanding of the chemical basis of the units of heredity.

By a curious coincidence, the same year 1865 was essentially the time at which Friederich Miescher first described a gummy substance that he extracted from cells of pus that was then associated with the nucleus of these cells and which he then called the nucleic acids. The function of that material was quite unknown to him, and it is quite clear that Mendel knew nothing of Miescher's work and Miescher knew nothing of Mendel's work and the connection between these observations would remain obscure for many years. It was, in effect, rediscovered only in 1900. Simultaneously three groups of workers either read his papers, or did similar experiments or both, and then to their chagrin had to report that this obscure worker 35 years earlier had anticipated their results. And that time is the origin of modern genetic science.

Very promptly after the first reappearance of the concept of the gene in modern scientific work, a physician, Professor of internal medicine Archibald E. Garrod observed that the inheritance of certain metabolic difficulties in the human also followed the laws of Mendelian segregation and he adduced the ypothesis that in these cases the way that the gene worked was to affect the production or the failure of production of specific enzymes in the metabolism of the organism. This anticipation also laid neglected for very many years, and I was quite surprised to hear directly from George Beadle that even at the time that he did his work in 1941 he was unaware of Garrod's findings and concepts, which were pointed out very shortly thereafter.

Over that leap of time there was the full development of what is called formal genetics, the description of the patterns on inheritance from one generation to another, but essentially totally lacking in any concept as to the chemical basis by which these characteristics could be transmitted. No one doubted that in the sperm and in the egg there were chemical substances in which were embodied the potentialities for development that were associated with the concept of gene, but as to their actual chemical constitution nothing could be said at that time. That association was first made in an experimental way by Oswald Avery and his colleagues MacLeod and McCarty. MacCarty lives today as a professor emeritus at the Rockefeller University in the Rockefeller Institute, and of course I am very proud of that since that is now the institution over which I have the privilege to preside.

In 1944, Avery and his group made a determination of the chemi-

cal composition of a substance that could be isolated from some strains of the bacterial species the pneumococcus that caused pneumonia and that this substance, when added to cells of other strains, would convert their hereditary characteristics from "type two" to "type three" or from "type one" to "type two". These types are simply the serological types which are used in the practical identification through specific antibodies of one form of pneumonia infection from another. But much to Dr. Avery's surprise, and to that of just about everybody else in the world, this substance, that had a very interesting biological specificity in being able to convert the biological behavior of one bacterial form to that of another, was not a protein, was not an enzyme, but proved to be a kind of the DNA that Miescher had isolated and described so many years before.

From 1944 on, there was little question but that the structure of DNA and how it functioned in cells might be the central question in experimental biology. At least so it appeared to me as a young student. But there were some difficulties about pursuing that question further. If one wished to study general biology and general genetics in Avery's system, you had not only the question of working with the pneumococcus, which one might think of as being a rather dangerous organism to deal with for routine experimentation, also rather difficult to grow in the laboratory and in other ways not an easy organism for experimental purposes, but even more seriously at that time there was no concept that bacteria had genes. They could not be crossed; you could not talk about the segregation of characteristics in the next hybrid generation. There was no easy way to correlate one's early findings on the alteration of behavior in bacteria with the very large body of general biological information that we had about other plant and animal species and no way to connect those findings with the mainstream of genetic analysis.

And so, as a young student very deeply excited by Avery's findings, it seemed to me that one would wish to do either or both of two things. The first was tried, and at that time was unsuccessful: to see if one could use DNA as isolated from cells in the way that Avery had done, but to do it with an organism already more familiar for purposes of genetic investigation. One might have wished to do it with a mouse or with Drosophila, which were then the best-known organisms for genetic investigation, but a fungus neurospora was at hand and seemed like an excellent experimental material for those

purposes. This indeed was the organism in which Beadle and Tatum had just recently developed, for studies in biochemical genetics, experimental systems in which one could look for particular kinds of mutations very much like the ones that Garrod had found in the human, but which in the fungus neurospora could be susceptible to experimental control in the laboratory in a very convenient way. For example, some of the mutations that Beadle and Tatum had developed in neurospora were deficient in the enzymes needed for the production of certain specific growth factors. So that although the original wild type neurospora organism could grow easily on a simple synthetic medium and produce its own specific growth factors - its amino acids, its vitamins, and so on- using only glucose as a carbon source, these mutant strains would be defective in their nutrition in various respects, and would therefore grow only if specific nutrient substances were added to the medium. This allowed not only for the definition of very precise examples of genetic change which could be associated with specific biochemical defects, but also provided a very powerful experimental tool for looking for new genetic forms in the cultures of the organism. One needed only to start with a culture - which might have millions or thousands of millions of cells in it - that was deficient for growth requiring a specific material, like the vitamin thiamine or like the amino acid leucine. And if even one cell were present that had restored to it the normal synthetic capacities of the original organism, that cell would be able to proliferate, to grow, could be very readily detected by inoculating large numbers of cells into the simple medium which would permit the wild type organism to grow, yet which would not permit the mutant organism to grow. Well, that was an elegant and entirely satisfactory experimental design, but it didn't word. An I don't take too many apologies for that particular experiment not working - it's only in the last year that by the use of rather specialized methods has it been possible to introduce DNA into these kinds of fungi in order to accomplish that result.

Well, that was disappointing, but that was only half of the possibilities that were presented by the challenge of Avery's discovery. The other half was to see whether perhaps bacteria had a richer life than had been attributed to them before, to see whether bacteria could be crossed. And once that question was put seriously, I did go through the literature, I consulted many experts, and I had to reach the conclusion that perhaps science had been somewhat hasty

in deciding that bacteria were devoid of a sexual process. The only critical evidence for this really was no different than that mentioned by van Leeuwenhoek in the year 1676 in the first discovery of bacteria. And when he first saw them under the microscope, he said these were tiny, globule-like things, that he believed they were living organisms because they would divide in two and divide in two again, but he could see no evidence of any sexual process. But in contrast to that he had a very graphic description by which protozoa not only divided in two, but also would mate with one another: "like dragon flies on the wing" was his attribution. I believe that it is the very graphic distinction that Leeuwenhoek himself made at the very foundations of microbiology that led to the deeply-held myth that bacteria differed from the rest of the living world in lacking a sexual process. That myth was also engrained in the official taxonomy of bacteria in 1875. Ferdinand Cohn put order into the bacterial world with his publication of the classification of different forms of bacteria, and he put them all under the heading of schizomycetes, the fungi that divide only by fission. And so, of course, who would question the myth when the very name of the group of organisms was to imply that they had only an asexual method of reproduction. In fact, however, critical evidence on this point was never produced - it was just that no reputable microbiologist had noticed a sexual process. It would be hard to see under any circumstances, and there is still great controversy about the very details of the so called "conjugation" in bacteria at the present time, and of course since nobody then was really looking for it, it would have been even more difficult to find.

This was not such an important matter until Avery presented his challenge that the very crux of the development of molecular biology would depend on there being a genetics of bacteria, and so this was what provoked a reinvestigation of that myth, and in just a few weeks time it was possible to demonstrate that bacteria could be crossed with one another using an experimental design very similar to the one I mentioned before concerning the efforts to transform neurospora. One simply took mutants of a bacterium - and here, with the help of Professor Tatum I used what has become a very famous organism, Escherichia coli strain K12. Mutants were obtained from it that possessed the same kind of nutritional defect mentioned before, and it was really child's play to demonstrate mixing cells that had different biochemical defects, that they would interact

with one another to produce normal type cells. Further studies left without any doubt that also in bacteria there was a well-developed genetic system.

This was also the foundation of all subsequent experimental studies using E. coli for this kind of molecular genetic investigation. Others deserve all of the credit for working out the details of the chemical foundations of that transfer and even for the direct demonstration that it is the transmission of DNA from the male cell to the female cell that is the crucial event in the conjugation process. I will skip very quickly over many important contributions to the further development of our present concepts, and even here I have to simply summarize very quickly a number of elements.

The relationship between DNA structure and protein structure received a very important boost by the study of a human genetic disease - and this should remind you again of Archibald Garrod. Only in this case the disease was an alteration of hemoglobin associated with sickle-cell disease, and it was in 1949 that Linus Pauling and his colleagues demonstrated that the disease which for some years had been known to be caught by an inherited Mendelian defect was associated with a chemical change in the structure of a protein, in the structure of hemoglobin. In 1953, also stimulated by the work of Avery and what had then followed by Hotchkiss and Chargaff, Watson and Crick worked out the fundamental details of the physico-chemical structure of DNA, the famous double helix that I believe nowadays everyone has heard about and possibly many readers could evey draw by heart. 1953 can be used as a very definite starting point for the modern era of DNA investigation since we were now clearly working with DNA as a chemical entity: not as an abstraction, not as a mathematical postulate, but a material that not only could be examined in the test tube as any other material substance but which could be subjected to the same methods of chemical analysis as had been developed for polymer chemistry and for organic chemistry in general. And of course there has been an explosion of effort since that time. Strangely enough, with all of this very rapid progress and development in our understanding of DNA and its biological importance, it was not until 1956 that we had the correct number for the chromosomes in the human species - another scandalous delay over what were the technical possibilities---had there just been sufficient motivation to look and a sufficiently critical approach to asking questions of great importance in human

biology.

By 1960, Arthur Kornberg had reached major milestones in his program to carry the study of the biochemistry of DNA to the step of defining the enzyme system which is responsible for that magical property by which DNA reproduces itself. Self-reproduction is of course the chemical propoerty underlying the very fact that DNA has genetic properties, that it can transmit information from parents to offspring and even from one species to another. This no longer seems so magical. We now understand that a DNA molecule acts as a template on which the newly growing DNA must be assembled correctly in order to be properly formed. Some of the details of these processes have been unraveled also thanks to distinguished biologists in Italy, notably thanks to important contributions by Arturo Falaschi, Vittorio Sgaramella and others in Pavia. They played an important role in the development of the enzymology which has been a necessary part of the leap from the scientific foundations of dealing with DNA as a chemical entity and as a substrate for enzymes in 1960 to the first, you might say, technological accomplishment of splicing together or joining together two DNA molecules from arbitrary origins in the test tube and thereby for the first time making DNA molecules under human design that occur rarely if they do occur at all under natural conditions.

It was still some years after that that we saw the first actual application of this knowledge to a problem in medicine. Dr. Y.W. Kan, on the foundations of the work that Pauling had started, really got after the DNA sequence - the actual structure of the DNA in human cells that do and do not have the genetic defect of the sickle cell hemoglobin - and demonstrated a change in the actual structure at that point, which is already beginning to be useful for diagnostic purposes to enable one to detect this disease under prenatal conditions, which had not been possible before.

And, of course, during this decade we have seen the further explosion of this engineering possibility of putting different kinds of DNA together at will in a convenient vector, and E. coli K12 continues to dominate the technology as well as the science of this because so much has been learned about it and because it is a very convenient organism. The basis of industrial and pharmaceutical application is not just that one can get the continued replication of a DNA introduced by design into a bacterial carrier, but also that under appropriate conditions that DNA can be expressed in the bac-

terium and produce the same protein which is its natural function in the cell from which that DNA was originally obtained. And from that, one keeps seeing almost every day new reports and new inventions of uses that can be made of this property of DNA for practical purposes.